

AL-TR-1992-0115
NMRI-92-74

AD-A262 580



**ACUTE DELAYED NEUROTOXICITY
EVALUATION OF TWO JET ENGINE
OILS USING A MODIFIED NAVY
AND EPA PROTOCOL**

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AUGUST 1992

FINAL REPORT FOR THE PERIOD JUNE 1991 THROUGH JUNE 1992

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TECHNICAL REVIEW AND APPROVAL

**AL-TR-1992-0115
NMRI-92-74**

The experiments reported herein were conducted according to the "Guide for the Care and Use of Laboratory Animals," Institute of Laboratory Animal Resources, National Research Council.

This report has been reviewed by the Office of Public Affairs (PA) and is releasable to the National Technical Information Service (NTIS). At NTIS, it will be available to the general public, including foreign nations.

This technical report has been reviewed and is approved for publication.

FOR THE COMMANDER



**JAMES N. McDOUGAL, Lt Col, USAF, BSC
Deputy Director, Toxic Hazards Division
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REPORT DOCUMENTATION PAGE			Form Approved OMB No. 0704-0118	
Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing the collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Washington Headquarters Services, Directorate for Information Operations and Reports, 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302, and to the Office of Management and Budget, Paperwork Reduction Project (0704-0188), Washington, DC 20503.				
1. AGENCY USE ONLY (Leave Blank)	2. REPORT DATE August 1992	3. REPORT TYPE AND DATES COVERED Final Report, June 1991-June 1992		
4. TITLE AND SUBTITLE Acute Delayed Neurotoxicity Evaluation of Two Jet Engine Oils Using a Modified Navy and EPA Protocol		5. FUNDING NUMBERS Contract F33615-90-C-0532 PE 62202F PR 6302 TA 630200 WU 63020002		
6. AUTHOR(S) E.R. Kinkead, R.E. Wolfe, S.A. Salins, C.S. Godin, C.D. Flemming, and G.B. Marit				
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES) ManTech Environmental Technology, Inc. P.O. Box 31009 Dayton, OH 45431 0009		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES) AL/OET Armstrong Laboratory Wright Patterson AFB, OH 45433-6573		10. SPONSORING/MONITORING AGENCY REPORT NUMBER AL-TR-1992-0115 NMRI-92-74		
11. SUPPLEMENTARY NOTES				
12a. DISTRIBUTION/AVAILABILITY STATEMENT Approved for public release; distribution is unlimited.			12b. DISTRIBUTION CODE	
13. ABSTRACT (Maximum 200 words) This study was initiated with three objectives. The first was to determine if either of two jet engine oils had the potential to produce delayed neuropathy. The major component of each oil is a mixture of hydrocarbon-based esters. One formulation contained 3% tricresylphosphate (TCP) isomer additive, whereas the second contained 3% of the ortho derivative of tricresylphosphate (TOTP), a known neurotoxicant. A second objective was to determine if the Navy repeated-high-dose of 420 mg/kg/day was sufficiently sensitive to determine neurotoxicity. The last was to compare the results of the former Environmental Protection Agency (EPA) limit test of 5 g/kg with the new standard of 2 g/kg. The assays performed indicated that the jet engine oil containing TOTP produced delayed neuropathy, whereas the jet engine oil containing TCP did not. Repeated dose levels greater than 420 mg/kg/day (1000 and 2000 mg/kg/day) produced organophosphorus-induced delayed neuropathy (OPIDN), whereas hens dosed at 420 mg/kg/day were asymptomatic. No potential for OPIDN was indicated in hens treated with a single dose of 2 g/kg, but hens dosed at the previous EPA limit dose had significant brain neurotoxic esterase (NTE) inhibition and axonopathy by 30 days posttreatment.				
14. SUBJECT TERMS Axonopathy Chickens Jet Engine Oil Formulation Neurotoxic Esterase		Neurotoxicity Tricresylphosphate Tri-o-tolyl Phosphate		15. NUMBER OF PAGES 39
17. SECURITY CLASSIFICATION OF REPORT UNCLASSIFIED		18. SECURITY CLASSIFICATION OF THIS PAGE UNCLASSIFIED		16. PRICE CODE
19. SECURITY CLASSIFICATION OF ABSTRACT UNCLASSIFIED		20. LIMITATION OF ABSTRACT UL		
NSN 7540 01-280-5500		Standard Form 298 (Rev. 2-89) Prescribed by ANSI Std Z39-18 298 102		

PREFACE

This is one of a series of technical reports describing results of the experimental laboratory programs conducted at the Toxic Hazards Research Unit (THRU), ManTech Environmental Technology, Inc., located at Wright-Patterson Air Force Base, Ohio. Work at the THRU is performed under Department of the Air Force Contract No. F33615-90-C-0532 (Study No. N06). Lt Col James N. McDougal served as Contract Technical Monitor for the Toxicology Division, Occupational and Environmental Health Directorate, Armstrong Laboratory.

This document serves as a final report on the in-life neurotoxicity of two jet engine oil formulations. The research described herein began in June 1991 and was completed in June 1992. This work was sponsored by the U.S. Navy under the direction of CAPT David A. Macys, MSC, USN, and was supported by the Naval Medical Research and Development Command Task MR04122201006. The opinions contained herein are those of the authors and are not to be construed as official or reflecting the view of the Department of the Navy or the Naval Services at large.

The animals used in this study were handled in accordance with the principles stated in the *Guide for the Care and Use of Laboratory Animals*, prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council, Department of Health and Human Services, National Institute of Health Publication #86-23, 1985, and the Animal Welfare Act of 1966, as amended.

The authors would like to acknowledge Susan Dille, Joanne Drerup, Richard Godfrey, and Janet Wilson for their excellent technical assistance. We also thank Ms. Mary Barth and the Mobil Environmental and Health Sciences Laboratory for their assistance in the preparation of the protocol for this study and for the analysis of hen brain neurotoxic esterase.

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ABBREVIATIONS

C	Celsius
cm	Centimeter
cm ³	Centimeter cubed
EPA	Environmental Protection Agency
F	Fahrenheit
ft	Feet
g	Gram
h	Hour
kg	Kilogram
mg	Milligram
min	Minute
mL	Milliliter
mm	Millimeter
mm Hg	Millimeter of mercury
N	Number
nm	Nanometers
NMRI/TD	Naval Medical Research Institute Detachment (Toxicology)
NTE	Neurotoxic esterase or neuropathy target esterase
OPIDN	Organophosphorous-induced delayed neuropathy
p	Probability
SEM	Standard error of the mean
TCP	Tricresylphosphate
THRU	Toxic Hazards Research Unit
TOCP	Triorthocresyl phosphate
TOTP	Tri- <i>o</i> -tolyl phosphate

SECTION 1

INTRODUCTION

Two formulations of jet engine oils, containing triarylphosphate additives, are of current interest to the Navy. One oil formulation contains 3% tricresylphosphate (TCP) whereas the other contains 3% tri-*o*-tolyl phosphate (TOTP) also known as triorthocresyl phosphate (TOCP), a known neurotoxic compound that is often used as a reference compound or positive control in acute delayed neurotoxicity studies done with phosphate esters. These oil samples could potentially cause axonal degeneration characteristic of organophosphate-induced delayed neurotoxicity (OPIDN) due to the arylphosphate ester additives.

Triarylphosphates such as TOTP are known esterase inhibitors and have been found to cause delayed neurotoxic effects in humans (Murphy, 1986). A single exposure to a neurotoxic organophosphorous compound can produce axonal damage after a delay of 8 to 10 days (Abou-Donia, 1981; Murphy, 1986). Nerve injury may occur in humans after chronic exposure at low doses. Cats and adult chickens have also demonstrated neurotoxic effects following exposure to these compounds (Beresford and Glees, 1963; Davis and Richardson, 1980; Abou-Donia, 1981; Bickford, 1984). Organophosphorous compounds that cause axonal pathology interact with the enzyme neurotoxic esterase (NTE) in the initial step of the delayed neurotoxicity (Johnson, 1975). This reaction occurs within hours of dosing and can be measured in brain tissue. Inhibition of NTE in the brain correlates with that in the spinal cord and nerve (Davis and Richardson, 1980; Caroli and Lotti, 1982).

The Navy has historically utilized a chicken bioassay to screen hydraulic fluids for OPIDN potential (MIL-H-19457B; (SH), 2 December 1963, Section 4.4.7). The bioassay required by this military specification details dose levels (maximum of 420 mg/kg), dosing regimen (5 daily treatments), and observation times for the assessment of OPIDN potential. The hydraulic fluids are typically composed of 100% organophosphates. The lubricant formulations in this study contained only 3% organophosphates. Whether the chicken bioassay is sensitive enough to detect the neurotoxic character of these substances is not known. An alternative assay, measuring the inhibition of brain NTE, has been used by other laboratories to estimate the potential for OPIDN. It has been shown that there is a correlation between an organophosphate compound's capacity to inhibit brain NTE by greater than 70% and the production of clinical signs of OPIDN (Lotti and Johnson, 1978, 1980). However, this threshold should not be considered absolute as incidences of brain NTE inhibition greater than 70% have occurred without clinically apparent neuropathy (Schwab and Richardson, 1986).

A study recently concluded in this laboratory (Kinkead et al., 1990) following MIL-H-19457B specifications, and using these organophosphate-containing oils, resulted in no clinical indication of OPIDN in the test hens treated at the maximum dose level of 420 mg/kg. An NTE assay performed by Mobil Oil Corporation found significant inhibition (greater than 70%) of brain NTE activity in hens treated at 420 and 1000 mg/kg of the jet engine oil containing 3% TOTP (Appendix A). The high-dose-level group (1000 mg/kg) was included for brain NTE assay only; none of the hens were maintained for posttreatment observation of clinical neuropathy. The results of the NTE assay, particularly on the high-level group, indicated that the doses specified in the military bioassay may not be high enough to demonstrate delayed neuropathy, which could result from ingestion of low levels of a neurotoxic aryl phosphate ester in an oil formulation.

The United States Environmental Protection Agency (U.S. EPA, 1991) has recently revised their Acute Delayed Neurotoxicity Guidelines to require a single-dose-level no greater than 2 g/kg (revised downward from 5 g/kg). There is some concern that a single-dose assay, at this dose level, will not be sensitive enough for some classes of chemicals to detect sufficient NTE inhibition, histopathology, or ataxia to identify weak neurotoxic agents.

The EPA has also included in their revised guidelines a requirement for an NTE assay to be performed in conjunction with the histopathology evaluation and clinical assay. Because the NTE assay was performed for the first time in this laboratory, brain tissue removed from the hens in this study were evenly divided between the Mobil laboratory and the THRU for obtaining NTE data. This afforded a comparison of results and validation of the THRU NTE procedure.

The objective of this research was threefold: first, to investigate the potential of the lubricants to produce OPIDN, and second, to compare the Navy's protocol with the single-dose EPA protocol on the *Delayed Neurotoxicity of Organophosphorus Substances Following Acute Exposure* (U.S. EPA, 1991). The Navy protocol has been in use longer than the EPA's, requires greater numbers of animals, and is more labor intensive. The EPA protocol requires fewer animals and fewer dose administrations than the Navy protocol. If the EPA protocol is proven to be as sensitive as the Navy protocol, it could possibly be considered for future use in assaying materials of interest to the Navy. The third objective was to determine if the change in the EPA limit test would alter its ability to identify potential neurotoxicants.

SECTION 2

MATERIALS AND METHODS

TEST MATERIAL

Jet Engine Oils (Supplied by Mobil Oil Corp.):

Naval Medical Research Institute/Toxicology Detachment (NMRI/TD) No. 91-141-2
Additive: 3% TCP
Density: 1.003 g/mL at 60 °F
Initial Boiling Point: >600 °F
Vapor Pressure: <0.1 mm Hg at 37.8 °C

NMRI/TD No. 91-140-1
Additive: 3% TGTP
Density: 1.003 g/mL at 60 °F
Initial Boiling Point: >600 °F
Vapor Pressure: <0.1 mm Hg at 37.8 °C

Positive and Negative Control Materials:

NMRI/TD No. 91-140-2
TOTP practical grade, Lot # A13B, supplied by Mobil Oil Corp., CAS #78-30-8.

NMRI/TD No. 91-141-1
TCP Stock 9550; Lot No. 5202J-5-A, supplied by Mobil Oil Corp., CAS #1330-78-5.

Corn Oil, CAS # 8001-30-7, commercial grade (Mazola®). The corn oil, purchased locally, was tested for the presence of peroxides prior to use.

ANIMALS

Delayed neurotoxicity potential was evaluated using leghorn hens (*Gallus domestica*, Carey Nick 320 hybrid, Carey Farms, Inc., Marion, OH) 8 to 14 months of age, weighing between 1.1 and 2.1 kg. The debeaked hens were identified by leg bands and group-housed in 3 ft x 6 ft pens to allow free movement. Food (MannaPro, Eggmaker 15 Crumbles) and water were provided ad libitum. Hens were maintained on a 15-h light/9-h dark cycle starting at 0300 h.

Verbal communication with the supplier (Carey Farms, Inc.) provided additional flock history and husbandry practices. Table 1 lists the vaccinations administered to the flock. No pesticides were applied to hens used in this study, nor were disinfectants used while the birds were in the poultry houses. When poultry houses became vacant, they were cleaned and disinfected with formaldehyde. The supplier indicated that the flock had not experienced any disease problems.

TABLE 1. FLOCK VACCINATION HISTORY^a

Vaccination	Age of Hen
Marek's disease	1 day
Infectious bronchitis	2 weeks
Infectious bursal disease	2 weeks
Newcastle disease	2 weeks
Infectious bronchitis (booster)	10-12 weeks
Newcastle disease (booster)	10-12 weeks
Fowlpox	20-24 weeks

^a Provided verbally by Cady Farms, Inc., Marion, OH.

DELAYED NEUROTOXICITY ASSESSMENT

Modified Navy Bioassay

This study was designed to meet the requirements for testing of acute delayed neurotoxicity as described in military specification MIL-H-19457B except that the treatment regimen was modified. Dose levels of 420, 1000, and 2000 mg/kg were used for the two test compounds, replacing standard dose levels defined in the specifications. A second positive control material (TCP) was also added to the treatment regimen. The dosing was as follows:

Engine Oils	Groups of four hens each treated with 2000, 1000, or 420 mg/kg/day for 5 days.
TOTP Positive Controls	Groups of four hens each treated with 60, 30, or 13 mg/kg/day for 5 days.
TCP Positive Controls	A group of four hens treated with 60 mg/kg/day for 5 days.
Corn Oil	A group of four hens given the maximum total volume of fluid equal to that given the test hens (i.e., 2.0 mL/kg/day).

Single-Dose Assay

Groups of eight hens were treated with a single dose of either 2000 mg test material/kg body weight (the present EPA limit dose) or 5000 mg/kg (the former EPA limit test). Four hens per group were used in an NTE assay, whereas the remaining hens were evaluated for clinical signs of OPIDN over a 30-day period. The dosing regimen was as follows:

Engine Oil/3% TOTP	Groups of eight hens each treated with either 5000 or 2000 mg/kg.
Engine Oil/3% TCP	A group of eight hens each treated with 2000 mg/kg.
TOTP Positive Controls	A group of eight hens each treated with 500 mg/kg.

Administration of Test Compounds

Test substances were administered to unfasted adult hens by oral intubation employing a 5-cm³ syringe fitted with a 5-cm infant feeding catheter. Each hen was weighed prior to the initial dose. Groups treated with the jet engine oils were administered neat material. The TOTP and TCP groups were administered the compound diluted in corn oil. Dilutions were prepared in a manner which resulted in each hen receiving a dose volume of 0.002 mL/g body weight.

Clinical Observations

Clinical observations and scoring began 7 days after the first dose and continued three times a week (Monday, Wednesday, and Friday) until 30 days after the initial dose. The following scoring system was used.

Symptom-free	0 points
Doubtful or minor symptoms	2 points
Positive paralytic symptoms	8 points
Advanced paralytic symptoms	12 points
Death	16 points

During clinical observations and scoring, the chickens were removed from their enclosures and placed on a rubber mat to provide sure footing. Symptoms observed in jet engine oil-treated hens were compared with those seen in the TOTP-treated hens during the posttreatment observation period. Calculated scores represented an average of the scores of three observers. The point scoring system, including definitions of symptoms can be found in Appendix B. The mean symptom scores noted on Day 21 after the initial dose were used to evaluate OPIDN potential.

Sacrifice and Histopathology

Hens that died during the 30-day study were examined for gross pathology at death. All surviving chickens were sacrificed (T61, IV) upon completion of the observation period and fixed via whole body perfusion. The entire brain, spinal cord, and both sciatic nerves (with attached gastrocnemius muscles), were collected for histopathologic evaluation. Histologic sections were prepared from the medulla, cerebellum, optic lobes, and frontal cortex of the brain; cervical, thoracic, and lumbosacral segments of the spinal cord; the proximal, middle, and distal thirds of one sciatic nerve; the entire gastrocnemius; and any observed gross lesions.

Neurotoxic Esterase (NTE) Assay

Additional hens (four per group) were included in the study to be used in the NTE assays. Doses were administered in the same manner as that described above for the modified Navy bioassay and for the single-dose assay. Twenty-four hours following the single dose or the fifth treatment of the repeated dose, all hens were euthanatized (T-61 iv) and the brain of each was removed for the NTE assay. The brain was divided into right and left hemispheres and each test laboratory (THRU and Mobil) received two right and two left hemispheres from each dose group.

Neurotoxic esterase activity at the THRU laboratory was measured using a microassay method (Correll and Ehrich, 1991). Brain tissue was homogenized in 150 volumes of buffer. Tissue samples were incubated with and without mipafox and/or paraoxon in microtiter wells. After incubation for 20 min, phenyl valerate was added as substrate and an additional incubation period of 15 min was provided for the reaction. The reaction was terminated by the addition of sodium dodecyl sulfate. Potassium ferricyanide was added to develop the color, which was read at 510 nm on a microplate spectrophotometer. Substrate and tissue blanks were included for each assay. Neurotoxic esterase activity was expressed as nanomoles of phenol formed per minute per gram of brain. Brain NTE inhibition was considered to be biologically significant if greater than 70% (Johnson and Richardson, 1984).

Statistical Analysis

Body weight plus or minus standard error of the mean (SEM) was calculated using a simple data description (BMDPID, Dixon, 1990). Body weights were compared using a one-factorial multivariate analysis of variance with Bonferroni multiple comparisons (SAS Institute, Inc., 1985). Fischer's Exact test and the Yates' Corrected Chi-Square test were used to compare histopathologic lesions (Zar, 1974). Severity of lesions were ranked and then compared using ANOVA and the Scheffe Multiple Comparison test (Zar, 1974). Neurotoxic esterase assays were evaluated utilizing a two-factorial analysis of variance with Bonferroni multiple comparisons (Barcikowski, 1983).

SECTION 3

RESULTS

Body Weight

Mean body weights of the hens during the course of the study are listed in Table 2. All repeat-dose hen groups, including the corn oil control group, had a slight, but not statistically significant, decrease in mean body weight during the posttreatment period. Only the low-dose TOTP hen group did not show a decrease in weight during the clinical observation period. The single-dose TOTP hens showed a statistically significant ($p < 0.05$) depression in weight when compared to the single-dose jet engine oil (91-140-1) group at Day 21.

TABLE 2. EFFECT OF ORAL INTUBATION OF JET ENGINE OILS ON CHICKEN BODY WEIGHTS (kg)^a

Treatment Group	Day 0	Day 7	Day 14	Day 21
Repeat Dose Regimen				
Corn Oil	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.2	1.5 ± 0.1
TCP (mg/kg)				
60	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.5 ± 0.1
TOTP (mg/kg)				
60	1.8 ± 0.2	1.6 ± 0.2	1.6 ± 0.2	1.4 ± 0.2
30	1.8 ± 0.1	1.6 ± 0.1 ^b	1.7 ± 0.1 ^b	1.6 ± 0.1 ^b
13	1.6 ± 0.1	1.6 ± 0.1	1.7 ± 0.2	1.7 ± 0.1
91-141-2 (mg/kg)				
2000	1.7 ± 0.1	1.6 ± 0.2	1.6 ± 0.1	1.4 ± 0.1
1000	1.6 ± 0.2	1.5 ± 0.1	1.6 ± 0.1	1.5 ± 0.1
420	1.7 ± 0.1	1.5 ± 0.1	1.4 ± 0.1	1.4 ± 0.1
91-140-1 (mg/kg)				
2000	1.8 ± 0.3	1.7 ± 0.3	1.8 ± 0.3	1.6 ± 0.2
1000	1.7 ± 0.1	1.7 ± 0.1	1.7 ± 0.1	1.6 ± 0.2
420	1.6 ± 0.3	1.6 ± 0.2	1.6 ± 0.2	1.5 ± 0.2
Single Dose Regimen				
91-141-2 (mg/kg)				
2000	1.7 ± 0.1	1.7 ± 0.2	1.8 ± 0.1	1.5 ± 0.2
91-140-1 (mg/kg)				
2000	1.7 ± 0.2	1.5 ± 0.2	1.6 ± 0.1	1.7 ± 0.1
TOTP (mg/kg)				
500	1.7 ± 0.1	1.5 ± 0.1	1.5 ± 0.1	1.3 ± 0.1 ^c

^a Mean ± SEM, N = 4; the 5000 mg/kg group was not weighed during the observation period.

^b N = 3.

^c Significantly different from the test hens that received the single dose TOTP containing oil (91-104-1) at $p < 0.05$ using a two-factorial analysis of variance.

Clinical Observations

Ataxia was observed in hen groups that received repeated doses of either 60 or 30 mg TOTP/kg and in the hen group treated with a single dose of 500 mg TOTP/kg (Table 3). The observations in these positive-control hens ranged from minor signs of ataxia (incoordination) to advanced paralytic symptoms. One hen of each repeated-dose group failed to show symptoms during the 30-day observation period. The four hens that received repeated doses of 60 mg TCP/kg showed no neurotoxic signs during the observation period.

TABLE 3. CLINICAL OBSERVATION SCORES OF TREATED HENS

Test Material	Dose (mg/kg)	Mean ^a Observation Score
Repeated Dose Regimen		
91-141-2	2000	0.3
91-141-2	1000	0.0
91-141-2	420	0.0
91-140-1	2000	3.0
91-140-1	1000	1.0
91-140-1	420	0.1
TOTP	60	4.7
TOTP	30	5.3 ^b
TOTP	13	0.0
TCP	60	0.0
Corn Oil	2000	0.0
Single Dose Regimen		
91-141-2	2000	0.0
91-140-1	5000	0.3
91-140-1	2000	0.0
TOTP	500	8.3

^a N = 4; individual animal clinical observation scores are given in Appendix C.

^b N = 3.

One hen from the 30 mg TOTP/kg group died following the fifth dose as a result of inhaling the TOTP/corn oil mixture. This hen was not replaced in the study.

Three of the four hens that received repeated doses of the high dose 91-140-1 oil (TOTP additive) showed signs ranging from minor (leg weakness in two hens) to positive paralytic symptoms (one hen). The fourth hen in this group was scored as having signs of leg weakness by one of three observers. Two of the four hens treated with repeated doses of 1000 mg 91-140-1/kg showed minor

neurotoxic signs. For the 420 mg/kg group, one hen was scored as having signs of leg weakness by one of three observers. Minor signs of incoordination were observed in one of four hens in the high-dose group treated with the 91-141-2 oil (TCP additive).

Neurotoxic Esterase Activity

Brain NTE activity of control and jet engine oil-treated hens were determined 24 h following the last treatment. The results are presented in Table 4. Statistically significant ($p < 0.01$) inhibition of brain NTE activity was observed in all hens treated with repeated doses of TOTP (63 to 89%), TCP (73/75%; THRU/Mobil), and jet engine oil with 3% TOTP (61 to 89%). Statistically significant inhibition of brain NTE activity was also noted in the hens treated with the two highest doses of the jet engine oil with 3% TCP (32-45%).

TABLE 4. EFFECTS OF TWO JET ENGINE OILS CONTAINING EITHER TCP OR TOTP ON BRAIN NEUROTOXIC ESTERASE ACTIVITY IN ADULT HENS FOLLOWING REPEATED OR SINGLE DOSE

Treatment Group	Daily Dose mg/kg/day	Total Organophosphates Administered (mg/kg)	Brain NTE Activity as % Change from Negative Control ^a	
Repeated Dose Regimen			THRU	Mobil
Jet Engine Oil 91-141-2 (TCP additive)	2000	300	-45 ^b	-32 ^b
	1000	150	-40 ^b	-36 ^b
	420	63	-21	-23
Jet Engine Oil 91-140-1 (TOTP additive)	2000	300	-89 ^b	-80 ^b
	1000	150	-70 ^b	-70 ^b
	420	63	-69 ^b	-61 ^b
TOTP (Positive Control)	60	300	-89 ^b	-87 ^b
	30	150	-79 ^b	-77 ^b
	13	65	-69 ^b	-63 ^b
TCP (Positive Control)	60	300	-73 ^b	-75 ^b
Single Dose Regimen				
Jet Engine Oil 91-141-2 (TCP additive)	2000	60	-5	-4
Jet Engine Oil 91-140-1 (TOTP additive)	5000	150	-95 ^b	--- ^c
	2000	60	-45 ^b	-42 ^b
TOTP (Positive Control)	500	500	-93 ^b	-91 ^b

^a Mean, N = 4.

^b Statistically different from respective control group at $p < 0.01$; no differences between assays using a two-factorial analysis of variance.

^c Brain NTE assay performed by THRU laboratories only.

A single dose of jet oil containing 3% TOTP resulted in statistically significant brain NTE inhibition at both treatment levels (95% and 45/42% for the 5000 and 2000 mg/kg groups, respectively). A single dose of jet oil containing 3% TCP (2000 mg/kg) resulted in minimal brain NTE inhibition. A positive control group that received a single dose of TOTP (500 mg/kg) showed brain NTE inhibition greater than 90%.

Necropsy

At necropsy, all hens, except one hen in an advanced paralytic state (from the 60 mg TOTP/kg group), were in good general condition. A gross lesion was observed in the liver of the one hen (2000 mg/kg repeated dose 91-141-2), which was described as a nodule with cysts containing a red fluid.

Histopathology

The histopathologic diagnosis for each tissue alteration, its corresponding incidence, and mean severity score are listed in Tables 5 through 8. The incidence data indicate the consistent occurrence of nervous tissue gliosis, perivascular cuffing, demyelination, and interstitial inflammation in all animal groups, including controls. Similar findings were noted for muscle alterations. The average severity scores for these lesions never exceeded 2.0 (slight) for any group.

Histologic lesions of statistical and morphologic significance were limited to the cervical or thoracic regions of the spinal cord. Demyelination and/or axonal degeneration and swelling (spheroids) were seen in the cervical cord of the 91-140-1 jet engine oil- (repeated-dosage groups of 2000 and 1000 mg/kg and the single-dose 5000 mg/kg group), TOTP- (single-dose and repeated 60, 30, and 13 mg/kg groups), and the TCP-treated hens. Similar significant lesions were observed in the thoracic spinal cords of single-dosed TOTP hens.

The hepatic lesion observed grossly corresponds to a nonneoplastic nodule of essentially normal liver tissue with blood-filled cyst-like spaces. This finding is not believed to be a treatment-related lesion and is most likely a congenital malformation. The hen sacrificed in advanced paralysis (clinical score of 12) was in a group that demonstrated significant histopathologic lesions of the spinal cord (60 mg/kg TOTP). However, the lesion that most likely explains the hen's clinical condition was severe axonal degeneration of the sciatic nerve. A lesion of similar severity was not observed in any other hen.

A summary of the OPIDN assay results is presented in Table 9.

TABLE 5. NEURAL HISTOPATHOLOGIC^a INCIDENCE SUMMARY (REPEATED ASSAY)

Material: Dose (mg/kg):	91-141-2			91-140-1			TOTP			TCP	Control
	2000	1000	420	2000	1000	420	60	30	13	60	2000
Animals on Study	4	4	4	4	4	4	4	4	4	4	4
Animals Necropsied	4	4	4	4	4	4	4	3	4	4	4
Brain											
Gliosis	0	1	1	1	2	0	3	1	0	1	2
Perivascular cuffing	3	1	1	3	3	4	0	3	2	2	1
Lymphocytic vasculitis	0	0	0	0	1	0	1	0	0	0	0
Cervical Spinal Cord											
Eosinophilic granules	1	0	0	0	1	0	0	0	0	0	0
Gliosis	0	0	0	0	0	0	0	0	0	0	1
Demyelination and axonal degeneration	1	0	0	4 ^b	3 ^b	1	4 ^b	3 ^b	3 ^b	1	0
Perivascular cuffing	1	3	1	3	2	2	1	2	1	1	0
Spheroids	1	1	0	3 ^b	3 ^b	1	4 ^b	3 ^b	2	3 ^b	0
Thoracic Spinal Cord											
Eosinophilic granules	1	0	0	0	2	1	0	1	0	0	0
Gliosis	0	0	0	0	1	0	1	0	0	1	0
Demyelination and axonal degeneration	1	0	0	1	1	0	1	1	0	0	0
Perivascular cuffing	0	1	1	0	2	2	2	2	2	1	0
Spheroids	2	2	0	3	0	1	3	2	0	1	1
Lumbosacral Spinal Cord											
Eosinophilic granules	2	2	3	2	3	3	2	2	2	2	1
Gliosis	1	0	0	1	1	0	1	0	0	1	0
Demyelination	1	0	0	0	0	0	0	0	0	0	0
Perivascular cuffing	0	1	1	0	1	0	0	2	0	2	0
Spheroids	0	0	0	0	2	0	1	0	0	0	0
Sciatic Nerve											
Demyelination and axonal degeneration	4	3	4	4	4	4	4	3	4	4	4
Inflammation, interstitial	3	3	3	3	4	1	2	3	0	4	3
Lymphocytic perivascularitis	1	2	1	2	2	0	0	1	1	1	0
Axonal degeneration	1	2	0	2	1	1	2	2	1	1	2
Schwann cell hyperplasia	2	0	0	2	2	1	1	1	0	1	1
Skeletal Muscle											
Inflammation, interstitial	1	1	1	3	2	2	1	1	1	3	3

^a A glossary of pathologic terminology is included in Appendix D.^b Statistically different from controls at $p < 0.01$.

TABLE 6. NEURAL HISTOPATHOLOGIC^a LESIONS AVERAGE SEVERITY^b SCORES (REPEATED ASSAY)

Material: Dose (mg/kg):	91-141-2			91-140-1			TOTP			TCP	Control
	2000	1000	420	2000	1000	420	60	30	13	60	2000
Animals on Study	4	4	4	4	4	4	4	4		4	4
Animals Necropsied	4	4	4	4	4	4	4	3	4	4	4
Brain											
Gliosis	0.0	0.3	0.5	0.3	0.8	0.0	1.0	0.3	0.0	0.3	0.8
Perivascular cuffing	0.8	0.3	0.5	1.0	0.8	1.3	0.0	2.0	0.5	0.8	0.5
Lymphocytic vasculitis	0.0	0.0	0.0	0.0	0.8	0.0	0.3	0.0	0.0	0.0	0.0
Cervical Spinal Cord											
Eosinophilic granules	0.3	0.0	0.0	0.0	0.3	0.0	0.0	0.0	0.0	0.0	0.0
Gliosis	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.5
Demyelination and axonal degeneration	0.3	0.0	0.0	1.8 ^c	1.0 ^c	0.3	2.0 ^c	1.7 ^c	0.8	0.5	0.0
Perivascular cuffing	0.3	1.0	0.3	1.0	1.0	0.5	0.3	1.3	0.3	0.3	0.0
Spheroids	0.3	0.3	0.0	1.5 ^d	1.3 ^d	0.3	2.3 ^c	1.0 ^d	0.5	1.0 ^c	0.0
Thoracic Spinal Cord											
Eosinophilic granules	0.3	0.0	0.0	0.0	0.5	0.3	0.0	0.3	0.0	0.0	0.0
Gliosis	0.0	0.0	0.0	0.0	0.3	0.0	0.3	0.0	0.0	0.3	0.0
Demyelination and axonal degeneration	0.3	0.0	0.0	0.3	0.3	0.0	0.3	0.3	0.0	0.0	0.0
Perivascular cuffing	0.0	0.3	0.5	0.0	0.8	0.5	0.5	1.0	0.5	0.3	0.0
Spheroids	0.5	0.8	0.0	1.0	0.0	0.3	0.8	1.0	0.0	0.3	0.3
Lumbosacral Spinal Cord											
Eosinophilic granules	0.5	0.5	0.8	0.5	0.8	0.8	0.5	0.7	0.5	0.5	0.3
Gliosis	0.3	0.0	0.0	0.3	0.3	0.0	0.3	0.0	0.0	0.3	0.0
Demyelination and axonal degeneration	0.3	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Perivascular cuffing	0.0	0.3	0.3	0.0	0.5	0.0	0.0	1.0	0.0	0.5	0.0
Spheroids	0.0	0.0	0.0	0.0	0.5	0.0	0.3	0.0	0.0	0.0	0.0
Sciatic Nerve											
Demyelination	1.3	0.8	1.3	1.5	1.8	1.8	1.8	2.0	1.3	1.0	1.5
Inflammation, interstitial	1.0	1.3	1.3	1.0	1.5	0.3	0.8	1.3	0.0	2.0	0.8
Lymphocytic perivascularitis	0.3	0.5	0.3	0.5	0.5	0.0	0.0	0.3	0.3	0.3	0.0
Axonal degeneration	0.3	0.5	0.0	0.8	0.3	0.5	1.0	1.3	0.3	0.5	0.5
Schwann cell hyperplasia	0.5	0.0	0.0	0.8	0.8	0.3	0.3	1.0	0.0	0.3	0.5
Skeletal Muscle											
Inflammation, interstitial	0.3	0.3	0.3	0.8	0.5	0.5	0.3	0.3	0.3	1.3	0.8

^a A glossary of pathologic terminology is included in Appendix D.

^b The scoring code for assessing lesion severity was progressively: 1—minimal, 2—slight (mild), 3—moderate, 4—significant, 5—severe.

^c Statistically different from controls at $p < 0.01$.

^d Statistically different from controls at $p < 0.05$.

TABLE 7. NEURAL HISTOPATHOLOGIC^a INCIDENCE SUMMARY (SINGLE-DOSE ASSAY)

Material: Dose (mg/kg):	91-141-2	91-140-1		TOTP	Control
	2000	5000	2000	500	2000
Animals on Study	4	4	4	4	4
Animals Necropsied	4	4	4	4	4
Brain					
Gliosis	1	0	2	1	2
Perivascular cuffing	3	2	3	2	1
Cervical Spinal Cord					
Gliosis	0	0	0	0	1
Demyelination and axonal degeneration	0	2	0	4 ^b	0
Perivascular cuffing	2	12	2	0	
Spheroids	0	2	0	4 ^b	0
Thoracic Spinal Cord					
Eosinophilic granules	0	0	1	1	0
Demyelination and axonal degeneration	0		0	3 ^b	0
Perivascular cuffing	1	2	2	1	0
Spheroids	0	1	2	4 ^b	1
Lumbosacral Spinal Cord					
Eosinophilic granules	3	1	3	3	1
Gliosis	0	0	1	2	0
Perivascular cuffing	1	2	2	2	0
Spheroids	0	0	0	2	0
Sciatic Nerve					
Demyelination	4	4	4	4	4
Inflammation, interstitial	4	4	3	2	3
Lymphocytic perivasculitis	0	0	2	2	0
Axonal degeneration	2	3	2	4	2
Schwann cell hyperplasia	0	3	2	2	1
Skeletal Muscle					
Inflammation, interstitial	1	4	1	1	3
Myositis	0	0	1	2	0

^a A glossary of pathologic terminology is included in Appendix D.^b Statistically different from controls at $p < 0.01$.

TABLE 8. NEURAL HISTOPATHOLOGIC^a LESIONS AVERAGE SEVERITY^b SUMMARY (SINGLE-DOSE ASSAY)

Material: Dose (mg/kg):	91-141-2	91-140-1		TOTP	Control
	2000	5000	2000	500	2000
Animals on Study	4	4	4	4	4
Animals Necropsied	4	4	4	4	4
Brain					
Gliosis	0.3	0.0	0.5	0.5	0.8
Perivascular cuffing	0.8	0.5	1.0	0.8	0.5
Cervical Spinal Cord					
Gliosis	0.0	0.0	0.0	0.0	0.5
Demyelination and axonal degeneration	0.0	0.5	0.0	2.3 ^c	0.0
Perivascular cuffing	0.5	0.3	0.8	0.5	0.0
Spheroids	0.0	0.8 ^d	0.0	2.0 ^c	0.0
Thoracic Spinal Cord					
Eosinophilic granules	0.0	0.0	0.3	0.3	0.0
Demyelination and axonal degeneration	0.0	0.3	0.0	0.8 ^c	0.0
Perivascular cuffing	0.3	0.5	0.8	0.5	0.0
Spheroids	0.0	0.3	0.5	1.3 ^c	0.3
Lumbosacral Spinal Cord					
Eosinophilic granules	0.8	0.3	0.8	0.8	0.3
Gliosis	0.0	0.0	0.3	0.5	0.0
Perivascular cuffing	0.3	0.5	0.5	0.8	0.0
Spheroids	0.0	0.0	0.0	0.5	0.0
Sciatic Nerve					
Demyelination	1.8	1.8	1.5	1.3	1.5
Inflammation, interstitial	1.5	2.0	1.0	0.8	0.8
Lymphocytic perivasculitis	0.0	0.0	0.5	0.5	0.0
Axonal degeneration	0.5	0.8	0.5	2.0	0.5
Schwann cell hyperplasia	0.0	1.0	0.5	0.8	0.5
Skeletal Muscle					
Inflammation, interstitial	0.3	1.5	0.3	0.3	0.8
Myositis	0.0	0.0	0.5	0.8	0.0

^a A glossary of pathologic terminology is included in Appendix D.

^b The scoring code for assessing lesion severity was progressively: 1—minimal, 2—slight (mild), 3—moderate, 4—significant, 5—severe.

^c Statistically different from controls at $p < 0.01$.

^d Statistically different from controls at $p < 0.05$.

TABLE 9. SUMMARY OF OPIDN ASSAY RESULTS

Group		Dose ^a	NTE ^b		Clinical Signs ^c	Histo-pathology ^d
			THRU	Mobil		
I	A	2000 (R)	-	-	-	-
Jet Oil	B	1000 (R)	-	-	-	-
(TCP)	C	420 (R)	-	-	-	-
II						
Jet Oil	A	2000 (R)	+	+	+	+
(TOTP)	B	1000 (R)	+	+	-	+
	C	420 (R)	-	-	-	-
III						
TOTP	A	60 (R)	+	+	+	+
	B	30 (R)	+	+	+	+
	C	13 (R)	-	-	-	+
IV						
TCP		60 (R)	+	+	-	+
V						
Corn Oil		2000 (R)	-	-	-	-
VI						
Jet Oil		2000 (S)	-	-	-	-
(TCP)						
VII						
Jet Oil		5000 (S)	+	N/A ^e	-	+
(TOTP)		2000 (S)	-	-	-	-
VIII						
TOTP		500 (S)	+	+	+	+

^a R = Repeated dose for 5 consecutive days; S = single dose.

^b NTE assay: + = inhibition greater than 70%; - = inhibition less than 70%.

^c Clinical signs: + = at least one hen per group with score of 8 or greater.

^d + = Histopathologic changes statistically significant from corn oil control hens.

^e Assay was not performed.

SECTION 4

SUMMARY

The lesions observed in this study that may occur as morphologic manifestations of OPIDN include demyelination, axonal degeneration, and myositis. These lesions may also occur as minimally to mildly severe background lesions. The demyelination and axonal degeneration observed in the thoracic and/or cervical spinal cords listed in the Results Section are interpreted to be a direct result of OPIDN based on statistical analysis of incidence or increased lesion severity. The severity scores of the lesions seen in the 91-140-1 hens fall in the range between the average severity scores of the high and medium TOTP-treated hens. Hens exposed to the low repeated dose of 91-140-1 did not show lesions that were statistically significantly different in incidence or severity from the controls. The remaining findings in all groups are considered to be background lesions.

No statistically significant clinical signs of OPIDN were observed in any of the hens treated (repeated or single treatment) with the jet engine oil containing 3% TCP (91-141-2) during the 30-day observation period. Treatment with this oil inhibited brain NTE no greater than 45%. Histopathologic evaluation of muscle and neural tissue at the conclusion of the observation period was unremarkable.

The jet engine oil containing 3% TOTP (91-140-1) produced ataxia, significant brain NTE inhibition, and demyelination and degeneration of axons at the highest repeated-dose level (2000 mg/kg). Although the repeated 1000 mg/kg group did not show unequivocal signs of incoordination or ataxia during the observation period (two hens were marginally positive), significant brain NTE inhibition and histopathology were apparent. These effects were similar to those found in the hen group that received a single dose of 5000 mg/kg (both groups had the same total organophosphates administered).

SECTION 5

DISCUSSION

This study was initiated with three main objectives. The first was to determine whether either of the jet engine oils had the potential to produce delayed neuropathy. The second was to determine if the Navy protocol (maximum dose level of 420 mg/kg/day) for evaluating delayed neurotoxicity was sufficiently sensitive to determine neurotoxicity in lubricants with relatively low levels of organophosphate. The third was to compare the results of the former single-dose EPA limit test of 5 g/kg with the new standard of 2 g/kg.

The assays performed in this study indicated that the jet engine oil with 3% TOTP produced delayed neurotoxicity. Significant depression of NTE, signs of ataxia, and significant axonopathy were noted at the higher dose levels of both the repeat-dose and the single-dose assays. The jet oil with 3% TCP was relatively nontoxic under the same treatment conditions.

This conclusion differs from that of the previous study (Kinkead et al., 1990) in which neither jet oil was considered to have neurotoxic potential based upon the standard Navy assay. However, with the additional data provided by the higher dose levels and the brain NTE enzyme results from pair-dosed hens, a reevaluation of the previous results is necessary.

The Navy protocol, using repeated doses over a five-day period, appears to be a more rigorous test than a single-dose regimen in regard to brain NTE inhibition. Greater inhibitions of brain NTE activity were obtained by the jet oils in the repeated-dose groups compared to the single-dose groups given an equivalent total organophosphate dose. For example, brain NTE depression in hens treated with either jet oil formulation following 5 days of oral administration at 420 mg/kg/day (2100 mg/kg total dose) was greater than the single 2000 mg/kg dose. An exception to this was the single dose 5000 mg/kg jet-oil-treated group, which had a 95% decrease in brain NTE activity.

The EPA single-dose limit test of 2000 mg/kg of the jet engine oil containing 3% TOTP indicated no potential for acute delayed neuropathy by clinical observation of treated hens, brain NTE inhibition, or by histopathologic evaluation of nerve and muscle tissues. However, hens treated with this jet engine oil at the former EPA single-dose limit level of 5000 mg/kg had biologically significant (>70%) inhibition of brain NTE activity at 24 h, and axonopathy at 30-days posttreatment.

In all cases where brain NTE activity was depressed by greater than 70%, significant histopathology (using criteria of both incidence and severity) was also observed (Table 9). Marginal axonopathy was noted in hens that received repeated doses of 13 mg TOTP/kg, whereas brain NTE was inhibited (69% THRU/63% Mobil). There appears to be a good correlation between brain NTE

inhibition and histopathologic findings. Clinical observations for incoordination and ataxia appear to be less sensitive than the end points of brain NTE depression or nervous tissue pathology, but still remained to be good indicators for predicting neurotoxicity.

The occurrence of background neural lesions in mature hens has been documented (Bickford and Sprague, 1982, 1984). Weakly pathogenic persistent viral infections may account for the background neural lesions. The frequent findings of lymphocytic inflammatory changes in the tissues examined in this study suggest a persistent viral infection.

SECTION 6

CONCLUSIONS

The results presented in this study have indicated that the 5-day multiple dose Navy protocol has similar sensitivity in predicting OPIDN potential as the single-dose EPA assay. To improve sensitivity, consideration should be made to include doses of at least 1000 mg/kg/day or a single bolus dose of 5 g/kg. Repeated treatment at that dose level would result in a total dose equivalent to the former EPA single-dose limit value. This study has shown that repeated doses produced greater brain NTE inhibition than single doses of similar total active organophosphate. Had a dose level of 1000 mg/kg/day been incorporated into the original acute delayed neurotoxicity evaluation performed on these two jet engine oils, significant axonopathy would have indicated that the oil formulation containing 3% TOTP had neurotoxic potential.

As previously stated, a good correlation exists between brain NTE inhibition and histopathologic lesions indicative of OPIDN. If the Navy considers revising the standard bioassay for screening OPIDN candidates, the incorporation of a brain or lymphocyte NTE activity assay should be considered. Several studies have demonstrated significant NTE activity in hen peripheral lymphocytes and have shown that brain and lymphocyte NTE activities are well correlated (Dudek and Richardson, 1980, 1982; Richardson and Dudek, 1983; Schwab and Richardson, 1986). An advantage of using lymphocytes to measure NTE is that assessment of OPIDN and NTE can be determined from the same animal and would provide a source of comparison to human lymphocyte data (i.e., Lotti and Johnson, 1978).

Based on the results of this study, the single-dose EPA protocol lacks the sensitivity of a 5-day multiple dose study to evaluate potential neurotoxic substances. In this particular case, the jet oil containing 3% TOTP would not have been considered as a potential OPIDN candidate if tested only at the revised EPA limit test level of 2000 mg/kg. However, the former limit test of 5000 mg/kg does indicate that the jet oil has neurotoxic potential. It is possible that neurotoxic agents may not be identified using the lower standard of 2000 mg/kg.

SECTION 7

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APPENDIX A

BRAIN NTE ACTIVITY FOLLOWING REPEATED ORAL GAVAGE OF TWO JET ENGINE OILS (ORIGINAL ASSAY)^a

Test Substance	Daily Dose (mg/kg/day)	Brain NTE Activity ^b (% Change from Negative Control)
Jet Engine Oil (contains 3% TCP)	1000	-45 ^c
	420	-38 ^c
	360	-29
	300	-32
	240	-24
Jet Engine Oil (contains 3% TOTP)	1000	-86 ^c
	420	-78 ^c
	360	-71 ^c
	300	-51 ^c
	240	-56 ^c
TOTP (positive control)	90	-80 ^c
	75	-84 ^c
	60	-85 ^c

^a Brain NTE activity performed by Mobil Environmental and Health Science Laboratory. Data were not included in technical report AAMRL-TR-90-018.

^b Mean of four hens per group.

^c Significantly different from corn oil control at $p < 0.05$ using Student's t-test.

APPENDIX B

GRADING SYSTEM FOR CLINICAL OBSERVATIONS^a

POINT SCORE SYSTEM

Symptom free	-	0 points
Doubtful or minor symptoms	-	2 points
Positive paralytic symptoms	-	8 points
Advanced paralytic symptoms	-	12 points
Death	-	16 points

DEFINITIONS

Doubtful or minor symptoms include leg weakness.

Positive paralytic symptoms includes lack of leg coordination (even though movement is free), loss of balance and a tendency to fall back on its rump due to leg weakness.

Advanced paralytic symptoms include inability to walk and hyperextension, ataxia, being completely prostrate, and moribundity.

^a From Navy specifications (MIL-H-19457B).

APPENDIX C

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING THE INITIAL PERORAL DOSE OF 91-141-2 (TCP ADDITIVE)

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060006	2000	0	0	0
N060010	2000	0	0	0
N060018	2000	2	2	0
N060028	2000	0	0	0
N060003	1000	0	0	0
N060007	1000	0	0	0
N060026	1000	0	0	0
N060044	1000	0	0	0
N060008	420	0	0	0
N060014	420	0	0	0
N060025	420	0	0	0
N060036	420	0	0	0

SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
THE INITIAL PERORAL DOSE OF 91-140-1 (TTP ADDITIVE)

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060017	2000	0	2	2
N060033	2000	2	2	2
N060035	2000	2	0	0
N060045	2000	8	8	8
N060016	1000	2	2	2
N060022	1000	0	0	0
N060023	1000	2	2	2
N060042	1000	0	0	0
N060027	420	0	0	0
N060029	420	0	0	0
N060030	420	2	0	0
N060034	420	0	0	0

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
THE INITIAL PERORAL DOSE OF TOTP**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060012	60	2	0	0
N060013	60	0	0	0
N060038	60	8	2	8
N060041	60	12	12	12
N060002 ^a	30	-	-	-
N060024	30	8	8	8
N060032	30	8	8	8
N060043	30	0	0	0
N060011	13	0	0	0
N060015	13	0	0	0
N060031	13	0	0	0
N060009	13	0	0	0

^a Died during treatment period, no observation scores available.

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
THE INITIAL PERORAL DOSE OF TCP**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060004	60	0	0	0
N060005	60	0	0	0
N060020	60	0	0	0
N060040	60	0	0	0

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
THE INITIAL PERORAL DOSE OF CORN OIL**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060001	2000	0	0	0
N060019	2000	0	0	0
N060021	2000	0	0	0
N060037	2000	0	0	0

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
A SINGLE PERORAL DOSE OF 91-141-2 (TCP ADDITIVE)**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060046	2000	0	0	0
N060047	2000	0	0	0
N060049	2000	0	0	0
N060059	2000	0	0	0

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
A SINGLE PERORAL DOSE OF 91-140-1 (TTP ADDITIVE)**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060003	5000	0	2	0
N060004	5000	0	0	0
N060005	5000	0	0	0
N060006	5000	0	0	2
N060053	2000	0	0	0
N060056	2000	0	0	0
N060057	2000	0	0	0
N060058	2000	0	0	0

**SCORING OF NEUROTOXIC EFFECTS OBSERVED 21 DAYS FOLLOWING
A SINGLE PERORAL DOSE OF TTP**

Animal Number	Dose (mg/kg)	21-Day Score		
		Observer 1	Observer 2	Observer 3
N060051	500	12	8	12
N060052	500	8	8	8
N060055	500	2	2	8
N060060	500	12	8	12

APPENDIX D

GLOSSARY OF PATHOLOGIC TERMS

BRAIN

Gliosis: An aggregation of any noninflammatory (glial) cell. A minimal lesion occupies 1 to 20% of a 40 x field and a mild lesion up to 40% of a 40 x field.

Perivascular cuffs: The presence of variable numbers of rows of lymphocytes surrounding blood vessels of the meninges or neuropil. Minimal severity is considered to be a cuff of 4 or fewer rows; mild, 5 to 8 rows; and moderate, 9 to 12 rows.

Vasculitis: The presence of inflammatory cells within the wall of a blood vessel. The severity is graded the same as perivascular cuffing.

SCIATIC NERVE

Axonal degeneration: A linear tract consisting of a dilated myelin sheath variably filled with eosinophilic material (axonal debris or myelin) and rare phagocytic cells. These digestion chambers are frequently bordered by a few Schwann cells. A minimal lesion is 1 tract per 20 x field, mild is 2 to 4 separate tracts, moderate is 5 to 7 tracts, and marked is 8 to 10 tracts per 20 x field.

Demyelination: The presence of small clear spaces (dilated myelin sheaths) containing small amounts of brightly eosinophilic material (myelin). Minimal severity is 1 to 3 separate parallel foci within a 20 x field, mild is 4 to 7 per field, and moderate is 8 to 12 per field.

Interstitial inflammation: Variable numbers of primarily lymphocytes, occasionally with fewer heterophils, outside the nerve proper. This includes the perineurium, surrounding soft tissues and associated vessels. A minimal lesion is confined within a 40 x field; mild, within a 20 x field; and moderate, within a 10 x field.

Perivasculitis: Variable numbers of lymphocytes surrounding blood vessels within the nerve. Severity is scored the same as perivascular cuffing in the central nervous system.

Schwann cell hyperplasia: Proliferation of cells often forming parallel rows surrounding a nerve fiber. A minimal lesion is 3 or fewer pairs of rows within a 20 x field, mild is 4 to 5 pairs of rows within a 20 x field, and moderate is 6 to 8 pairs within a 20 x field.

SKELETAL MUSCLE

Interstitial inflammation: Essentially the same as in the sciatic nerve. It includes the endo-, epi-, and perimysium surrounding soft tissues and associated vessels. Severity is scored the same as in the sciatic nerve. Inflammation coursing between muscle fibers or bundles will be minimal unless there is interstitial expansion.

Myositis: Presence of inflammatory cells within myocytes or significant myodegeneration associated with predominately interstitial inflammation. Severity is scored the same as for inflammation.

SPINAL CORD (all segments)

Axonal swelling (spheroids): 1 spheroid within a 20 x field is considered a minimal lesion, 2 to 3 is mild, and 4 to 6 moderate.

Demyelination and axonal degeneration: The presence of dilated myelin sheaths mixed with variable amounts of eosinophilic debris (myelin or neuronal debris) and scattered phagocytic (Gitter) cells. A minimal lesion occupies less than 10% of the thickness of the white matter; mild, up to 33%; and moderate, up to 50%. If the lesion involves to some extent more than one white tract, it is considered to be diffuse.

Eosinophilic granules: Variable numbers of small bright red granules within neurons.

Gliosis: See BRAIN.

Perivascular cuffs: See BRAIN.

QUALITY ASSURANCE

The study, "Acute Delayed Neurotoxicity Evaluation of Two Jet Engine Oils Using a Modified Navy and EPA Protocol," was conducted by the ManTech Environmental Technology, Inc., Toxic Hazards Research Unit under the guidance of the Environmental Protection Agency's Good Laboratory Practice Standards, 40 CFR 792. No claim will be made that this was a "GLP" study as no attempt was made to adhere to the strict requirements of those standards.

The various phases of this study were inspected by members of the Quality Assurance Unit. Results of the inspections were reported directly to the Study Director at the close of each inspection.

DATE OF INSPECTION

ITEM INSPECTED

November 14, 1991	Animal dosing for Navy and EPA phases.
November 19, 1991	Animal dosing for NTE phase.
November 20, 1991	Animal observation and scoring for Navy and EPA phases.
November 22, 1991	Sacrifice and specimen collection for EPA and NTE phases.
December 12, 1991	Sacrifice and specimen collection for Navy phase.
February 7, 1992	Sacrifice and specimen collection for amendment #1.
February 19, 1992	Animal observation and scoring for amendment #1.
June 22-23, 1992	Data and final report audit.
August 13, 1992	Final report audit.

The Quality Assurance Unit has determined through review process that this report accurately describes those methods and standard operating procedures required by the protocol and that the reported results accurately reflect the raw data obtained during the course of the study. No discrepancies were found that would alter the interpretations presented in this Final Report.

M. G. Schneider

M. G. Schneider
QA Coordinator
Toxic Hazards Research Unit

Date 13 August 1992

**END
FILMED**

DATE:

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